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Antibiotic Susceptibility Profile and Survival of *Bifidobacterium adolescentis* and *Bifidobacterium catenulatum* of Human and Avian Origin in Stored Yoghurt

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ABSTRACT

Bifidobacteria are categorized as health-promoting microorganisms (probiotics) in the gastrointestinal tracts of humans and animals. Antibiotic susceptibility is a key criterion for probiotic agent selection. Good survival of probiotics during storage at selected storage temperature(s) is highly desirable. Bifidobacteria isolated from human and chicken were thus characterized phenotypically by their ability to produce fructose-6-phosphoketolase enzyme. Eight selected isolates were then characterized on molecular basis employing genus-specific and species-specific 16S rRNA gene primers, and their antibiotic susceptibilities were also evaluated. Isolates were confirmed to be strains of *B. catenulatum* (4) and *B. adolescentis* (4). Studied strains were resistant to streptomycin, gentamycin, cloxacillin and cotrimoxazole but susceptible to chloramphenicol, augmentin, amoxicillin and erythromycin. Three strains (*B. catenulatum* HM2, *B. catenulatum* HM3 and *B. catenulatum* HM5) showed atypical tetracycline resistance. *B. catenulatum* HM2, *B. adolescentis* CH2 and *B. adolescentis* CH3 showed significant reduction in counts ($p < 0.05$) and survived in adequate amount in yoghurt stored at 4°C and -18°C for 4 weeks. In addition to presenting acceptable antibiotic susceptibility profile and exhibiting good survival in stored yoghurt, the bifidobacteria isolates inhibited *Staphylococcus aureus* ATCC 25925 and *Escherichia coli* ATCC 25922 *in-vitro* and demonstrated potential for application as probiotic agents for human and agricultural purposes.

Keywords: *Bifidobacterium*, probiotics, 16S rRNA, survival at low temperature.

Introduction

Bifidobacterium species are reported to present evidence of distinct immunomodulatory effect in-vitro and in-vivo (Chenoll *et al.*, 2011; Fujiwara *et al.*, 2004; Isolauri *et al.*, 2000; Reid *et al.*, 2003). Poupard *et al.* (1973) concluded, after elaborate revision of investigation reports on bifidobacteria and the better health of breast-fed infants, that bifidobacteria aid in better nutrition of infants and, thus, indirectly contributes to greater resistance to infection. The popularity of bifidobacteria in many commercial probiotic products can be traced to their beneficial effects such as antagonism against other microorganisms, anti-mutagenic and anti-carcinogenic properties, anti-inflammatory, cholesterol reduction activities, and general well-being of host (Pereira and Gibson, 2002; Wagner *et al.*, 1997; Xiao *et al.*, 2006a; Xiao *et al.*, 2006b).

The increasing application of viable microorganisms also goes with the increasing probability of negative health impact through the use of wrong probiotic agents. Classical methods of identification and classification of microorganisms usually involve cultivation on selective media, colony analysis, and microscopic examination, followed by biochemical tests. This protocol is known to be

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prone to error, variability, and it is time consuming. Other taxonomic tools that are conventionally used include bacteriophage typing, serotyping and antibiotic susceptibility patterns. These also have their peculiar limitations. In bifidobacteria taxonomic work, the demonstration of the enzyme fructose-6-phosphate phosphoketolase in cell-free extract of bifidobacteria has been used as confirmation for *Bifidobacterium* species (Kimura *et al.*, 1997). Currently, molecular techniques are being favoured widely for confirmatory identification of microorganisms including bifidobacteria (Biavati *et al.*, 1982; Duranti *et al.*, 2013; Matsuki *et al.*, 2002; Wendy and Lynne, 1998). One of such techniques is the use of genus-specific and species-specific 16S rRNA gene primers in bacteria identification (Fujimoto and Watanabe, 2013; Matsuki *et al.*, 2002; Wendy and Lynne, 1998).

The issue of safety (Donohue and Salminen, 1996; Ishibashi and Yamazaki, 2001) brings to fore the need for accurate identification of probiotics, usually accomplished through molecular methods (Langendijk *et al.*, 1995; Matsuki *et al.*, 1999; Satokari *et al.*, 2001; Scardovi and Crociani, 1974; Ventura *et al.*, 2001). A more specific safety concern is the issue of antibiotic resistance transfer from probiotics to other intestinal microbes. Minimizing this possibility in probiotic product development is a major prerequisite. The issue of safety of probiotics, like that of other internally consumed products, continues to receive emphasis. For instance while possession of antibiotic resistance is sought after in probiotic combined therapy with antibiotics, the fear of resistance-gene transfer to other intestinal microorganisms appears real. The existence and activities of mobile DNAs such as transposons, retrotransposons, plasmids and viruses in microbes have been reported (Climo *et al.*, 1996; Grohmann *et al.*, 2003). So far no mobile DNA on antibiotic resistance has been confirmed in bifidobacteria to our knowledge. Only atypical tetracycline resistance gene tet (W) have been observed (Masco *et al.*, 2006; Moubareck *et al.*, 2005). Available reviews and research reports severally concluded that bifidobacteria are safe for human and animal use (Donohue and Salminen, 1996; Ishibashi and Yamazaki, 2001; Masco *et al.*, 2006; Moubareck *et al.*, 2005).

Probiotics are often included in fermented milks, infant formulas, cheese, ice cream and related products to improve their health beneficial properties. The value and benefits of bifidobacteria are yet to be enjoyed in sub-Saharan African countries, including Nigeria. The focus of this study was to isolate, characterize, and evaluate *Bifidobacterium* species that can be used as probiotic agents in humans and animal agricultural practices.

Materials and Methods

Bacteria culture

The cultures used in this study were isolated from faecal samples of adult human and intestinal content of chicken using the *Bifidobacterium* medium of Nebra and Blanch (1999). The Oxoid anaerobic system (Oxoid jar with Oxoid AnaeroGen: O2 below 1%, CO2 9% – 13%) was used throughout in this project when anaerobic incubation was required. Isolates were stored in sterile Oxoid MRS broth with 20% glycerol at -20°C when long period of storage was required (Monaghan *et al.*, 1999).

Phenotypic identification

Isolated gram positive catalase negative rods were further identified phenotypically by demonstration of fructose-6-phosphate phosphoketolase. This test was performed based on specified method (Zinedine and Faid, 2007).

DNA isolation

DNA extraction was performed using the method of Alander *et al.* (1999) with modification. Cells were harvested from 24 h MRS broth culture (1.5 ml) by centrifuging at 13,000 rpm for 15 min. Cells were washed twice in normal saline by centrifuging (Eppendorf 5415R refrigerated centrifuge, Eppendorf, Germany), 200 µl of solution containing 10 mm Tris-HCl (pH 8) and 2.5 mg/ml of lysozyme was added, and content subsequently vortexed. Tubes were incubated at 37°C for 2h, 400 µl of lysis buffer (50mM Tris-HCl, 100mM EDTA,

1% SDS, pH 8) and 1mg/ml of proteinase k was added. Tube content was mixed and incubated in a water bath at 50˚C for 1h. This was followed with addition of 500 µl of phenol:chloroform (1:1). After mixing, debris was removed by centrifuging at 13000 rpm for 15 min at 4˚C (Eppendorf 5415R refrigerated centrifuge, Eppendorf, Germany). The supernatant was transferred to a sterile tube and extracted with chloroform: isoamyl alcohol (24:1) by centrifuging at 13000 rpm for 15 min. Supernatant was transferred to another tube and 50 µl 5M NaCl and 1 ml absolute ethanol added. Contents were kept overnight on ice to precipitate. Tube content was centrifuged, supernatant decanted and pellets allowed to dry. Dried pellets were dissolved in 50 µl of sterile water. The DNA extracts were stored at -20˚C.

Primers

Sequences highly conserved in the genus *Bifidobacterium* as designed by Matsuki *et al.* (1999 and 2002) using the 16S rRNA sequences were used by Biomers (Germany) to synthesize group-specific and species-specific gene primers. The specificity of the primers used (Table 1) in this identification had been tested and confirmed previously (Matsuki *et al.*, 1999; Matsuki et al., 2002). One genus-specific gene primer (g-BIFID) and 7 species-specific gene primers (*B. angulatum* BIANG, *B. adolescentis* BIADO, B. bifidum BIBIF, B. breve BIBRE, *B. catenulatum* group BICAT, *B. longum* BILON and *B. dentium* BIDEN) were applied for molecular identification of the 8 selected isolates. Lyophilized primers were dissolved in sterile purified water following the manufacturer's (Biomers, Germany) instructions.

Polymerase Chain Reaction (PCR)

Amplification was carried out based on Matsuki *et al.* (1999 and 2002) method. Each 25 µl PCR mixture composed of 10 mM Tris-HCl (pH 8), 50 mM KCl, 1.5 mM MgCl2, 0.2mM of each deoxynucleotide triphosphate, 0.25µM primer, 2 µl template DNA and 1U Taq DNA polymerase. Amplification program was one cycle of 94˚C for 5 min, 40 cycles of 94˚C for 30s, 48˚C for 30s, 72˚C for 1 min, followed by one cycle of 72˚C for 5 min using the Eppendorf Master Cycler Gradient (Eppendorf, Germany).

Table 1: Primers used

	Primer	Primer sequence	Reference
Bifidobacterium (group)	g-BiFID-F	CTCCTGGAAACGGGTGG	Matsuki *et al.*, 2002
	g-BiFID-R	GGTGTTCTTCCCGATATCTACA	
B. adolescentis	BiADO-F	CTCCAGTTGGATGCATGTC	Matsuki *et al.*, 1999
	BiADO-R	CGAAGGCTTGCTCCCAGT	
B. angulatum	BiANG-F	CAGTCCATCGCATGGTGGT	Matsuki *et al.*, 1999
	BiANG-R	GAAGGCTTGCTCCCCAAC	
B. bifidum	BiBIF-F	CCACATGATCGCATGTGATTG	Matsuki *et al.*, 1999
	BiBIF-R	CCGAAGGCTTGCTCCCAAA	
B. breve	BiBRE-F	CCGGATGCTCCATCACAC	Matsuki *et al.*, 1999
	BiBRE-R	ACAAAGTGCCTTGCTCCCT	
B. catenulatum group	BiCAT-F	CGGATGCTCCGACTCCT	Matsuki *et al.*, 1999
	BiCAT-R	CGAAGGCTTGCTCCCGAT	
B. longum	BiLON-F	TTCCAGTTGATCGCATGGTC	Matsuki *et al.*, 1999
	BiLON-R	GGGAAGCCGTATCTCTACGA	
B. dentium	BiDEN-F	ATCCCGGGGGTTCGCCT	Matsuki *et al.*, 1999
	BiDEN-R	GAAGGGCTTGCTCCCGA	

Electrophoresis

After amplification, the reaction mixtures were subjected to 1% (w/v) agarose gel electrophoresis in Tris-acetate/EDTA buffer, visualized with ethidium bromide/bromophenol blue staining under UV light (High Performance UV Transilluminator, Upland, USA) and DNA bands captured with canon power shot A590 camera. 1Kb DNA ladder (O'GeneRuler) was used as marker.

Antibiotic susceptibility test

The agar overlay disc method was used (Huys et al., 2002). Isolates were grown anaerobically at 37˚C for 24 h in MRS broth supplemented with 0.05% cysteine HCl, 1mg/l thymine and 1mg/l riboflavin. MRS agar was prepared, poured on plates and allowed to solidify. Soft MRS agar (3 ml) cooled to about 40˚C was inoculated with 200 µl of 24 h broth culture of isolates and spread on solidified MRS agar plates. Soft agar was prepared by adding 7.5 g/l Oxoid bacteriological agar into MRS broth. Inoculated plates were allowed to solidify. Antibiotic discs (Abtek Biologicals) were placed on inoculated plates which were then incubated at 37˚C for 24 h in an anaerobic atmosphere. Disc diameter was 6 mm. Antibiotics used were cotrimoxazole 25 µg, chloramhpenicol 10 µg, cloxacillin 5 µg, erythromycin 5µg, gentamicin 10 µg, augmentin 30µg, streptomycin 10 µg, tetracycline 10 µg, chloramhpenicol 30 µg and amoxicillin 25 µg.

Survival of isolates at low temperature in yoghurt

Yoghurt was produced using Richard (2001) methods with modification. Full cream milk powder and granulated sugar were purchased from a Lagos market. Two hundred and ten grams (210 g) of milk powder and 40 g of granulated sugar were dissolved in 1L sterile distilled water, pasteurized at 95˚C for 10 min, cooled to 44˚C and inoculated with yoghurt starter culture – *S. thermophilus*, *L. bulgaricus* and *L. acidophilus* (Yogomet, Germany). Cooled inoculated milk was distributed into 500 ml flasks after thorough mixing with magnetic stirrer. Flasks and contents were incubated in a water bath at 42 – 43˚C and pH monitored until pH dropped to 4.5. Produced yoghurt was pasteurized at 85˚C for 15 min, refrigerated overnight and divided into two. Subsequently, one portion was inoculated with test *Bifidobacterium* isolate culture (HM2, CH3, or CH2) as an adjunct to produce the probiotic yoghurt. Samples (pasteurized plain yoghurt and probiotic yoghurt) were stored in the fridge (4˚C) and freezer (-18˚C). The pH, taste and survival of isolates were monitored periodically during storage. MRS agar supplemented with 0.05% cysteine HCl, 1mg/l thymine and 1mg/l riboflavin was used for survival check. Statistical analysis of data was done using the Duncan Post Hoc tests and descriptive statistics.

Results and Discussion

Bifidobacteria obtained from human (5) and chicken (3) were anaerobic gram positive non-spore forming rods, catalase negative, produced lactic and acetic acid without gas from glucose fermentation in Oxoid MRS broth. Isolates were phenotypically identified to genus level through the production of fructose-6-phosphoketolase enzymes and then presumptively identified to species level using their carbohydrate fermentation patterns (results not shown). Authentication of identities of isolates at genus and species level was performed by the application of 16S rRNA-gene-targeted group and species-specific gene primers technique (Tables 1 and 2). Some PCR-amplified genes are shown in Figures 1 and 2.

Susceptibility of isolates to some antibiotics is reported in Table 3. Three isolates (*B. catenulatum* HM2, *B. adolescentis* CH2, and *B. adolescentis* CH3) were used to assess ability of strains to survive low storage temperatures (4°C and -18°C). Results are as represented in Figure 3 and Figure 4. *Strain B. catenulatum* HM2 and *B. adolescentis* CH3 did not record significant reduction in count up to day 14 of storage in yoghurt at 4°C. A decrease of about 38.51% and 47.63% occurred at day 30 of storage in yoghurt at 4°C for *B. catenulatum* HM2 and *B. adolescentis* CH3 respectively. For strain *B.*

adolescentis CH2 there was no significant reduction in number up to day 11 when stored in yoghurt at 4°C. However, a decrease in number by 38.16% at day 14, 68.84% day 22 and 76.86% at day 30 was recorded at this storage temperature. When stored at -18°C *B. catenulatum* HM2 exhibited a progressive reduction in number from 2.96 x 10^6 at day 0 to 1.55 x 10^6 at day 30.

Table 2: Isolates reaction with group and species-specific primers

Isolate	g-BIFID	BiADO	BiANG	BiBIF	BiBRE	BiCAT gp	BiLON	BiDEN	Identification
HM1	+	-	-	-	-	+	-	-	*B. catenulatum*
HM2	+	-	-	-	-	+	-	-	*B. catenulatum*
HM3	+	-	-	-	-	+	-	-	*B. catenulatum*
CH1	+	+	-	-	-	-	-	-	*B. adolescentis*
HM4	+	+	-	-	-	-	-	-	*B. adolescentis*
CH2	+	+	-	-	-	-	-	-	*B. adolescentis*
HM5	+	-	-	-	-	+	-	-	*B. catenulatum*
CH3	+	+	-	-	-	-	-	-	*B. adolescentis*
Product (bp)	**549-563**	**279**	**275**	**278**	**288**	**285**	**831**	**387**	

+ = positive reaction (amplification), – = no reaction (no amplification)

Table 3: Antibiotic susceptibility of bifidobacterial isolates (zones in mm)

Strain	COT	TET	STR	AUG	GEN	ERY	CXC	CHL1	CHL2	AMX
B. catenulatum HM1	-	12	-	20	8	15	-	18	25	20
B. catenulatum HM2	-	6	-	20	-	20	-	20	30	26
B. catenulatum HM3	-	7	-	20	-	18	-	20	36	25
B. adolescentis HM4	-	18	-	25	-	16	-	20	28	21
B. catenulatum HM5	-	6	-	20	-	20	-	20	30	26
B. adolescentis CH1	-	12	-	25	6	20	6	22	38	15
B. adolescentis CH2	-	18	-	24	-	20	6	20	32	28
B. adolescentis CH3	-	18	-	24	-	20	6	20	32	28

COT = cotrimoxazole 25 μg, CHL1 = chloramphenicol 10 μg, CHL2 = chloramphenicol 30 μg, CXC = cloxacillin 5 μg, ERY = erythromycin 5 μg, GEN = gentamicin 10 μg, AUG = augmentin 30 μg, STR = streptomycin 10 μg and TET =tetracycline 10 μg, AMX = Amoxycillin 25 μg.

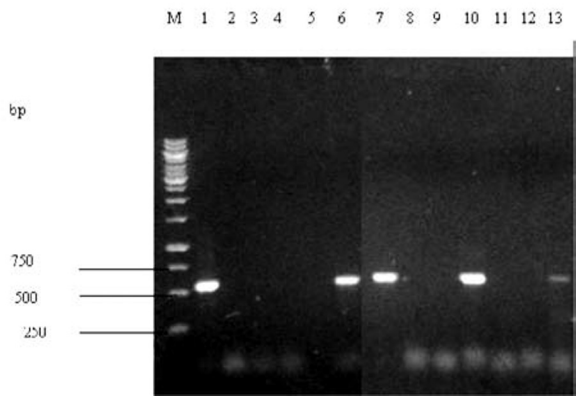

Fig 1: Agarose gel electrophoresis of amplified *Bifidobacterium* genus gene

M = marker, LANE 1 B. *catenulatum* HM1, LANE 6 *B. catenulatum* HM2, LANE 7 *B. catenulatum* HM3, LANE 10 *B. adolescentis* CH1, LANE 13 *B. adolescentis* HM4

Though *B. adolescentis* CH2 maintained its count up to day 11, there was a subsequent high reduction on day 14 when stored in yoghurt at -18^{6} C. However, a reasonable number of this strain remained until day 30 of storage in yoghurt at this temperature. Strain *B. adolescentis* CH3 decreased by 67.14% during storage at -18^{o}C in yoghurt for the 30 days investigation period. Despite the foregoing, strains maintained high counts after 4 weeks in yoghurt stored at the two temperatures (Figure 3 and Figure 4). The lowest count after 30 days storage in yoghurt was 1.55 x 10^{6} cfu/ml for *B. catenulatum* HM2 at -18^{o}C.

The specificity of the primers used in this study were previously tested and confirmed by Matsuki *et al.* (1999 and 2002). The results obtained attest to their reports. Distinct bands were only obtained with the corresponding species primer, indicating high specificity, even though 7 different species-specific gene primers were applied to each isolate. The DNA of the eight isolates whose identity were molecularly confirmed, amplified with the *Bifidobacterium* genus gene primer g-BIFID. B. *catenulatum gp* and *B. adolescentis*, though closely related phenotypically, were specifically identified molecularly as different species in this study as previously documented.

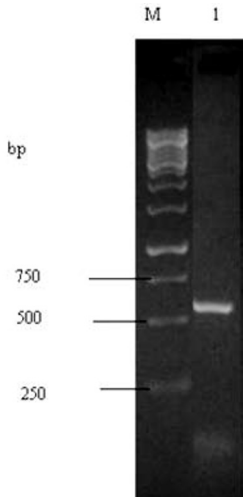

Fig. 2: Agarose gel electrophoresis of amplified genus gene

M = marker (1kb DNA ladder), lane 1 *B. adolescentis* CH2

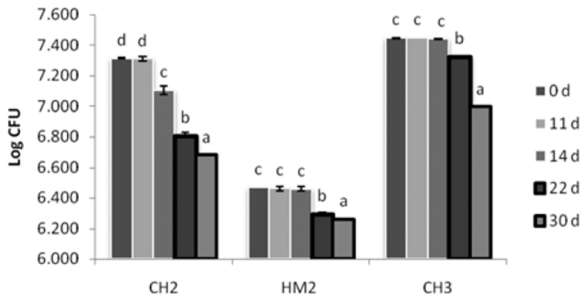

Fig. 3: Survival of bifidobacteria in yoghurt at 4^{o}C

CH2 = *B. adolecentis* CH2, HM2 = *B. catenulatum* HM2, and CH3 = *B. adolecentis* CH3

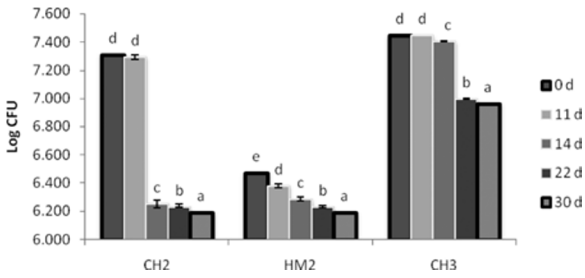

Fig 4: Survival of bifidobacteria in yoghurt at -18^{o}C

CH2 = *B. adolecentis* CH2, HM2 = *B. catenulatum* HM2, and CH3 = *B. adolecentis* CH3

Values with the same letter (for an isolate) do not significantly differ ($p > 0.05$)

Isolates susceptibility to nine antibiotics were tested. All isolates were resistant to streptomycin. This agrees with the result of Yazid *et al.* (2000) who concluded that bifidobacterial resistance to streptomycin appeared to be genus characteristic. In this study, resistance to gentamycin appears also to be a genus-wide phenomenon. Isolates that showed slight susceptibilities (6 mm – 8 mm) can be described as resistant as their zones of inhibition are very close to the diameter of the disc (6 mm). Masco *et al.* (2006) and Yazid *et al.* (2000) concluded that all their tested bifidobacteria strains were gentamicin-resistant. Other authors also reported strain resistance to gentamicin and streptomycin (Mättö *et al.*, 2007; Mayrhofer *et al.*, 2007). Resistance to aminoglycosides in anaerobes is seen as natural due to the absence of cytochrome-mediated drug transport system which prevents the drug from reaching its target (Bryan *et al.*, 1979; Bryan and Kwan, 1981).

All isolates were inhibited by chloramphenicol, augmentin and erythromycin. Kheadr et al. (2007), Masco *et al.* (2006) and Yazid *et al.* (2000) concluded that all bifidobacteria tested were susceptible to chloramphenicol and erythromycin. This study revealed all isolates to be resistant to cotrimoxazole. Cotrimoxazole is a combination of trimethoprim and sulphamethoxazole. Masco *et al.* (2006) observed general resistance to sulphamethoxazole and variable zones for trimethoprim for tested bifidobacteria. But they noted that the strains that exhibited susceptibility to trimethoprim showed only partial inhibition zones. The resistance of studied isolates to cotrimoxazole is therefore in agreement with these authors. The sulphonamides are said to inhibit cells by interfering with folic acid metabolism. They inhibit cells that synthesize their own folic acid for growth but will not interfere with the growth of cells which require preformed folic acid (Pelczar and Chan, 1981).

Variable zones were observed for tetracycline. This was also the conclusion of Yazid *et al.* (2000). Masco *et al.* (2006) also recorded that resistance to tetracycline was strain specific. Chloramphenicol and tetracycline are broad-spectrum antibiotics. Atypical tetracycline resistance was exhibited by 3 isolates – *B. catenulatum* HM2, *B. catenulatum* HM3 and *B. catenulatum* HM5. Other authors have also recorded this phenomenon in related studies of bifidobacteria susceptibility to antibiotics (Delgado *et al.*, 2008; Lim *et al.*, 1993; Moubareck *et al.*, 2005). Further studies by past authors to identify the bifidobacteria gene responsible for this tetracycline resistance observed the presence of an acquired non-plasmid tet(W) gene which was not integrated in the conjugative transposon TnB1230 (Florez *et al.*, 2006; Masco *et al.*, 2006). Mayrhofer *et al.* (2011), after primer based PCR studies of antibiotic resistance genes, concluded that dominant *Bifidobacterium* species from human intestine frequently harbour acquired tetracycline resistance encoded by a tet(W) gene.

In the present study, the eight isolates exhibited resistance to cloxacillin. Yazid *et al.* (2000) reported that strains differ in their sensitivity to cloxacillin. Kheadr *et al.* (2007) documented susceptibility of studied bifidobacterial strains to cloxacillin. Augmentin (amoxicillin + clavulanic acid) and amoxicillin inhibited all isolates in this study. Yazid *et al.* (2000) reported all tested 18 bifidobacteria strains from 10 species were sensitive to amoxicillin. Masco *et al.* (2006) concluded a genus susceptibility to amoxicillin for *Bifidobacterium*. Susceptibility to these β-lactam antibiotics is expected since there is no published report to our knowledge of detection of β-lactamase activities in *Bifidobacterium* species to date. Authors have reported bacterial resistance to β-lactam antibiotics (Kim *et al.*, 1998). Bacterial resistance to β-lactam antibiotics (penicillins and cephalosporins) is by production of β-lactamases which catalyses the hydrolysis of the β-lactam ring (Bush, 1989; Sanders and Sanders, 1992). Lee and Jeong (2002) studied the presence of phenotypic resistance and the genotyping of β-lactamase in some gram negative bacteria and observed occurrence of more than one β-lactamase gene in some 45% of the isolates. These enzymes confer broad spectrum resistance to penicillin and cephalosporin and are

said to often be plasmid-mediated (Paterson *et al.*, 2001). Research for β-lactamase inhibitors led to the discovery of clavulanic acid (Rolinson, 1991). Clavulanic acid on its own is a weak antibacterial but potent β-lactamase inhibitor and an effective potentiator of antibacterial activity of penicillins and cephalosporins as in augmentin (Buckland *et al.*, 1977). With respect to acquired antibiotic resistance and probiotic safety, the bifidobacteria isolates in this study appear risk-free since only few demonstrated atypical resistance to tetracycline possibly not based on acquired transmissible gene as have been previously reported in past investigations (Masco *et al.*, 2006; Moubareck *et al.*, 2005).

Technological consideration requires that selected probiotic organisms be produced, incorporated into food products or processed into usable forms and stored for variable periods before consumption. Previous investigations have documented good survival of species of *Lactobacillus* in yoghurt (Onyibe *et al.*, 2010) and soymilk (Onyibe *et al.*, 2009) stored at low temperatures. Ability of probiotic agents to survive at low temperatures in yoghurt was assessed in this study. Incorporated bifodobacteria cultures (*B. adolescentis* CH3, *B. catenulatum* HM2, *B. adolescentis* CH2) remained viable and were counted in adequate amount during storage at 4°C and -18°C for 4 weeks.

The good survival of studied strains in yoghurt at low storage temperatures further confirm that probiotic bifidobacteria can be packed and stored in usable food forms. Quality and effective probiotics applied in these forms are expected to maintain viability and impart no negative attributes to such products (Heller, 2001; Sarkar, 2008) as was observed in this study. The total bacteria, yeast/mould and coliform count obtained during quality monitoring of stored yoghurt and probiotic yoghurt in the present study collaborated the report of other researchers that probiotic cultures in food products can improve the microbiological quality of foods. No coliform was detected, and the bacteria and mould counts were lower compared to those obtained in the plain yoghurt samples without probiotic cultures (results not shown). In their evaluation of bifidobacteria for the production of antimicrobial compounds and assessment of performance in cottage cheese, O'Riordan and Fitzgerald (1998) reported significant reduction to no detectable level of *Pseudomonas* in cheese stored at 4°C on day 14 in the presence of *B. longum*, *B. breve* and *B. infantis*. These strains manifested inhibition against Pseudomonas in laboratory tests and extended the inhibitory activities in stored product even at low temperature, supporting the claim that probiotic organisms can be applied as preservative and quality control agents in manufactured products.

Conclusion

In addition to presenting acceptable antibiotic susceptibility profile and exhibiting good survival in stored yoghurt, the bifidobacteria isolates evaluated in this study inhibited *Staphylococcus aureus* ATCC 25925 and *Escherichia coli* ATCC 25922 *in-vitro* (results not shown), and demonstrated other potential for application as probiotic agents for human and agricultural purposes.

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